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(54) Title: THE USE OF ANTIBODIES TO TNF OR FRAGMENTS DERIVED THEREOF AND XANTHINE DERIVATIVES FOR COMBINATION THERAPY AND COMPOSITIONS THEREFOR

(57) Abstract

A combined preparation for simultaneous combined, simultaneous separate, or sequential use in the therapy or prophylaxis of disorders associated with undesirably high levels of TNF, e.g. septic or endotoxic shock and immunoregulatory and inflammatory disorders, which comprises an antibody to TNF or a TNF binding fragment thereof and a xanthine derivative. Particular preferred xanthine derivatives are 3,7-dimethyl-1-(5-oxo-hexyl)xanthine (known as Pentoxifylline or Trental) and 1-(5-hydroxy-5-methylhexyl)-3-methylxanthine and similar compounds. The anti-TNF antibody or fragment is preferably monospecific especially a humanised recombinant antibody or fragment. The ratio of xanthine derivative to anti-TNF antibody component used may be in the range between 450:1 and 1:10 and doses of anti-TNF component in the range 0.001-30mg/kg/day and doses of xanthine derivative in the range 0.5 to 100mg/kg/day may be administered during treatment of human or animal subjects. It has been found that when an anti-TNF antibody and a xanthine derivative are used together in some experimental models of septic shock, a surprising combination effect is observed.

The use of antibodies to TNF or fragments derived thereof and xanthine derivatives for combination theraphy and compositions therefor.

Field of the Invention

The present invention relates to a pharmaceutical product for the treatment of conditions associated with elevated levels of tumour necrosis factor-α (herein referred to as TNF) and to the manufacture of such a product. The pharmaceutical product may, for example, be employed in the treatment of sepsis, and, in particular in the treatment of septic, or endotoxic shock.

Background to the Invention

TNF is a cytokine which is produced by activated macrophage and other cells and is an important regulator in inflammation and immunity. It is implicated in septic shock - an often fatal condition associated with Gram-negative or Gram-positive bacteremia - as well as in other conditions such as adult respiratory distress syndrome, graft-versus-host disease and auto-immune diseases.

Treatments of these conditions involving the use of antibodies to TNF (anti-TNF antibodies) have been proposed and tested. For example, Beutler et al (Science (1985), 229, 869-871) showed that passive immunisation with a rabbit polyclonal antiserum against TNF protected mice from the lethal effects of Gram-negative endotoxin. Similarly, antibody therapy against TNF has been shown to decrease mortality in mice undergoing graft-versus-host disease (GVHD) and to prevent splenomegaly, and cutaneous and intestinal lesions associated with acute phase GVHD. (Piguet, P.F., et al, J. Exp. Med. 1987; 166: 1280; Shalaby, M.R., et al, Transplantation 1989; 47: 1057).

In addition, patent applications of Celltech Limited disclose the use of anti-TNF antibodies in the amelioration of side effects associated with anti-lymphocyte therapy of graft-rejection (WO89/08460), and with anti-neoplastic chemotherapy (WO89/01950).

Certain xanthine derivatives are also known to be inhibitors of TNF. For example, EP-A-0344586 of Hoechst Aktiengesellschaft discloses xanthine derivatives effective in inhibiting the TNF whose release is induced by certain TNF-releasing substances such as amphoterocin B.

The present inventors have observed that in some experimental models of septic shock a surprising combination effect is produced if an antibody to TNF and a xanthine derivative are used in combination.

Summary of Aspects of the Invention

Thus, according to a first aspect of the present invention there is provided a pharmaceutical product comprising an antibody to TNF or a TNF binding fragment thereof and a xanthine derivative as a combined preparation for simultaneous combined, simultaneous separate, or sequential use in therapy.

Such a pharmaceutical product may take the form of a pharmaceutical composition in which the antibody to TNF, or TNF binding fragment thereof and the xanthine derivative occur in admixture, optionally together with a pharmaceutically acceptable excipient, diluent, or carrier.

The ratio by weight of xanthine derivative to anti-TNF antibody in the composition may vary between 450: 1 and 1:10; preferably it is in the range 150:1 - 1:5 and particularly preferably between 30:1 and 1:2 for example 1:1.

In particular the preferred xanthine derivatives for inclusion in the pharmaceutical product of the present invention are pentoxifylline (3,7-dimethyl-1-(5-oxohexyl)xanthine), also known as Trental, and 1-(5-hydroxy-5-methylhexyl)-3-methyl xanthine which is referred to hereafter as HWA 138.

The anti-TNF antibody or TNF binding fragment thereof included in the pharmaceutical product of the present invention is preferably a TNF neutralising antibody or antibody fragment. By neutralisation is intended the reduction in, or inhibition of a biological activity of TNF as measured by an in vitro or in vivo test.

The anti-TNF antibody or fragment included in the pharmaceutical product of the present invention may in general belong to any immunoglobulin class. Thus for example the anti-TNF antibody may be an immunoglobulin G or immunoglobulin M antibody.

The anti-TNF antibody may be of animal, for example mammalian origin and may be for example of murine, rat or human origin. The antibody may be a whole immunoglobulin, or a fragment thereof, for example a fragment derived by proteolytic cleavage of a whole antibody, such as $F(ab')_2$, Fab' or Fab fragments, or fragments obtained by recombinant DNA techniques, for example Fv fragments (as described in International Patent Application No. WO89/02465).

The anti-TNF antibody may be polyspecific but is preferably monospecific for human TNF. The antibodies may be polyclonal or monoclonal antibodies. Particularly useful antibodies for use according to the invention include recombinant anti-TNF antibodies and fragments thereof, i.e. anti-TNF antibodies or fragments which have been produced using recombinant DNA technqiues.

Especially useful recombinant antibodies include, (1) those having an antigen binding site at least part of which is derived from a different antibody, for example those in which hypervariable or complementarity determining regions of one antibody have been grafted into variable framework regions of a second, different, and preferably human, antibody (as described in European Patent Application EP-A-239400); (2) recombinant antibodies or fragments wherein non-Fv sequences have been substituted by non-Fv sequences from other, different antibodies (as described in European Patent Applications EP-A-171496, EP-A-173494 and EP-A-194276); or (3) recombinant antibodies or fragments possessing substantially the structure of a natural immunoglobulin but wherein the hinge region has a different number of cysteine residues from that found in the natural immunglobulin, or wherein one or more cysteine residues in a surface pocket of the recombinant antibody of fragment is in the place of another amino acid residue present in the natural immunoglobulin (as described in International Patent Applications Nos. WO89/01974 and WO89/01782 respectively).

The anti-TNF antibodies may be prepared using well-known immunological techniques employing TNF as antigen. Thus, for example, any suitable host may be injected with TNF and the serum collected to yield the desired polyclonal anti-TNF antibody after appropriate purification and/or concentration, (for example by affinity chromatography using immobilised TNF as the affinity medium). Alternatively, splenocytes or lymphocytes may be recovered from the TNF-injected host and immortalised using for example the method of Kohler et al, Eur. J. Immunol. 6, 511, (1976), the resulting cells being diluted and cloned to obtain a monoclonal line producing anti-TNF antibodies in accordance with conventional practice. Antibody

fragments may be produced using conventional technique for example by enzymatic digestion of whole antibodies e.g. with pepsin [Parham, J. Immunol., 131, 2895, (1983)] or papain [Lamoyi and Nisonoff, J. Immunol. Meth., 56, 235, (1983)].

Where it is desired to produce recombinant anti-TNFα antibodies these may be produced using, for example, the general methods described in the above-mentioned patent specifications.

According to a second aspect of the invention there is provided the use of an antibody to TNF and of a xanthine derivative in the manufacture of a pharmaceutical product of the first aspect of the invention.

For example, an antibody to TNF and a xanthine derivative as described above may be mixed together and a pharmaceutically acceptable excipient, diluent, or carrier may optionally also be mixed in.

The pharmaceutical product may be utilised in any therapy where it is desired to reduce the level of TNF present in the human or animal body. The TNF may be in circulation in the body or present in an undesirably high level localised at a particular site in the body.

For example, elevated levels of TNF are implicated in immunoregulatory and inflammatory disorders and in septic, or endotoxic, and cardiovascular shock. The pharmaceutical product according to the first aspect of the present invention may be utilised in therapy of conditions which include sepsis, septic or endotoxic shock, cachexia, adult respiratory distress syndrome, AIDS, allergies, psoriasis, T.B., inflammatory bone disorders, blood coagulation disorders, burns, rejection

episodes following organ or tissue transplant and autoimmune disease e.g. organ specific disease such as thyroiditis or non-specific organ diseases such as rheumatoid and osteo-arthritis.

Additionally, the pharmaceutical product may be used to ameliorate side effects associated with TNF generation during neoplastic therapy and also to eliminate or ameliorate shock related symptoms associated with the treatment or prevention of graft rejection by use of an antilymphocyte antibody, or may be used for treating multi-organ failure (MOF).

The pharmaceutical product according to the first aspect of the invention is preferably for treatment of sepsis, or septic/endotoxic shock.

The pharmaceutical product according to the first aspect of the invention may be for administration in any appropriate form and amount according to the therapy in which it is employed. It may be for prophylactic use, for example where circumstances are such that an elevation in the level of TNF might be expected or alternatively, the product may be for use in reducing the level of TNF after it has reached an undesirably high level or as the level is rising.

The pharmaceutical product may take any suitable form for administration, and, in particular, will be in a form suitable for parenteral administration e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion it may take the form of a suspension, solution or emulsion of all or each of the components is an oily or aqueous vehicle and it may contain formulatory agents such as suspending, stabilising and/or dispersing agents.

Alternatively, the product may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Where the anti-TNF and xanthine derivative components of the pharmaceutical product are for separate administration each may be formulated according to conventional practice and the formulation of each component may contain one or more other active ingredients.

In particular, while the anti-TNF antibody is likely to be unsuitable for oral administration such a limitation may not apply to the xanthine derivative.

When the xanthine derivative is for oral administration the formulation may contain, in addition, to the active ingredient, additives such as; starch - e.g. potato, maize or wheat starch or cellulose - or starch derivatives such as microcrystalline cellulose; silica; various sugars such as lactose; magnesium carbonate and/or calcium phosphate. It is desirable that, if the formulation is for oral administration it will be well tolerated by the patient's digestive system. To this end it may be desirable to include in the formulation mucus formers and resins. It may also be desirable to improve tolerance by formulating the xanthine derivative in a capsule which is insoluble in the gastric juices. It may also be preferable to include the xanthine derivative in a controlled release formulation.

In a still further aspect of the invention there is provided a method of treatment of a human or animal subject suffering from or at risk of a disorder associated with an undesirably high level of TNF, the method comprising administering to the subject an effective amount of the pharmaceutical product according to the